



A new species of dink frog (Anura: Eleutherodactylidae: *Diasporus*) from Cordillera de Talamanca, Costa Rica

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Abstract

A new dink frog species of the genus *Diasporus* (Eleutherodactylidae) is described from the Cordillera de Talamanca in Costa Rica. The new species shows an amazing polymorphism in the dorsal and ventral coloration that varies from red to black and white that is unique in the genus. Here we include the description of its mating call, sexual dimorphism relative to coloration, and comments on its distribution and conservation status. In addition, molecular data that complement the morphological taxonomy are shown.

Key words: *Diasporus ventrimaculatus* sp nov, intraspecific variation, polymorphism, polychromatism, Costa Rica

Resumen

Una nueva especie de rana campana del género *Diasporus* (Eleutherodactylidae) es descrita de la Cordillera de Talamanca en Costa Rica. La nueva especie muestra un sorprendente polimorfismo en la coloración dorsal y ventral que varía entre rojo hasta blanco y negro, lo cual es único en el grupo. Aquí incluimos la descripción de su canto de cortejo, dimorfismo sexual relacionado con la coloración así como comentarios sobre su distribución y estatus de conservación. Se muestran, además, datos moleculares que complementan la taxonomía morfológica.

Palabras claves: *Diasporus ventrimaculatus* sp nov, variación intraespecífica, polimorfismo, policromatismo, Costa Rica

Introduction

Costa Rica hosts one of the best known herpetofaunas of any tropical country (Savage 2002). However there are still some areas within Costa Rica that have received little attention from herpetologists, such as the mountains of the Peninsula de Nicoya, the north Caribbean region and the highlands of the Cordillera de Talamanca. New species of salamanders (García-París *et al.* 2008; Wake *et al.* 2007), anurans (Lips 1996; Lips & Savage 1996) and reptiles (Lips & Savage 1994; Savage & Guyer 1998; Savage & Lahanas 1989) were recently described for the Talamancas, based on material collected from the few surveys carried out in the last decades. These new discoveries highlight the incomplete nature of our herpetological knowledge, especially in highland areas.

In a recent survey of a high elevation valley known as Valle del Silencio, on the Caribbean versant of the Cordillera de Talamanca (Fig. 1), we found a remarkably abundant dink frog from the genus *Diasporus* (*sensu* Hedges *et al.* 2008). Frogs in the genus *Diasporus* were formerly placed in the family Leptodactylidae under the genus *Eleutherodactylus* (Frost 1985), but recently Heinicke *et al.* (2007) divided the eleutherodactylines

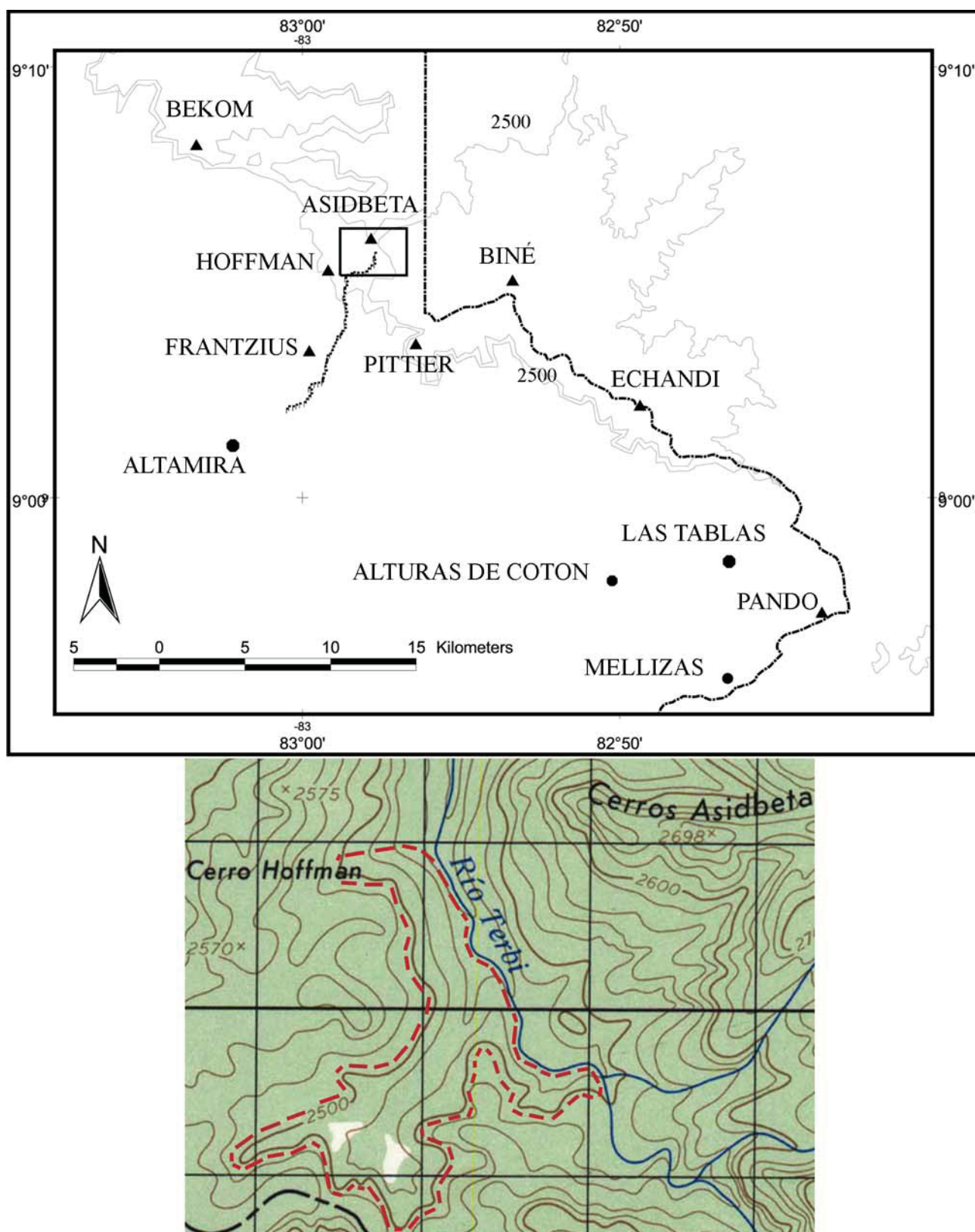


FIGURE 1. Map of the Valle del Silencio area showing the Altamira-Valle del Silencio route (dotted line). Also shown are additional sites sampled during past expeditions. In the lower part is a close-up of the Valle del Silencio with the potential distribution of *Diasporus ventrimaculatus* marked with the red dot line.

in to several genera based on a molecular phylogeny. The *Eleutherodactylus diastema* group (comprised by *E. diastema*, *E. vocator*, *E. hylaeformis*, *E. quidditus*, *E. gularis*, *E. tinker*, *E. scolodiscus* and *E. chalceus*) was reassigned to the genus *Pristimantis*, a group composed of approximately 400 species of South American origin (Heinicke *et al.* 2007). More recently, Hedges *et al.* (2008) added new sequences to the data set of Heinicke *et al.* (2007) and reorganized the classification of the “eleutherodactylinae”. Hedges *et al.* (2008) revealed that the frogs of the *Eleutherodactylus diastema* group formed a monophyletic clade with closer evolutionary affinities to the Caribbean clade of “eleutherodactylinae”. For this reason, Hedges *et al.* (2008) assigned the *Eleutherodactylus diastema* group (with the exception of *P. scolodiscus* and *P. chalceus*) to the genus *Diasporus*, characterized by the presence of a oval palmar tubercle, tympanic membrane usually differentiated, absence of cranial crests, digital discs expanded, finger I shorter than finger II and two longitudinal gular folds in males. *Pristimantis chalceus* and *P. scolodiscus* were found to be imbedded in the South American clade containing *Pristimantis*, and these species differ from *Diasporus* frogs by the presence of bifid palmar tubercles, weakly developed vomerine teeth and by not having the longitudinal gular folds (i.e. Lynch 2001; Hedges *et al.* 2008).

Diasporus contains few recognized species at present, but according to some authors (Lynch, 2001; Savage 2002), some taxonomic names may be obscuring greater diversity within the clade. Costa Rica is home to four of the eight species of this genus (Hedges *et al.* 2008), one of which, *Diasporus hylaeformis*, lives in the humid lower montane region between 1500 to 2500 meters of altitude (Savage 2002). This species commonly has the dorsal surfaces colored pink or diffuse red. However, the pink frog we found in Valle del Silencio presents significant variation in the dorsal and ventral coloration, from red to black and white and others morphological and ecological traits that indicate that this animal was a new species of *Diasporus*. Herein we present a description of this new species with notes on its natural history.

Materials and methods

Field-work was carried out on the east side of the Cordillera de Talamanca close to the Costa Rica-Panama border within Parque Internacional La Amistad between July and September 2008. During the surveys we made a 14 km transect from Altamira (9.0296 N, -83.0080 W, 1391 m elevation) on the Pacific slope to El Valle del Silencio on the Caribbean slope (9.1116 N, -82.9617 W, 2550 m; Fig 1). On the Pacific slope, the first three kilometers of the transect were in secondary forest that, according to locals we talked to, was only recently not dedicated to agriculture. The rest of the transect went through a mature oak forest that classified as Tropical Lower Montane Rainforest (Holdridge 2000) with a dry season between December and March (Herrera 1985). The Valle del Silencio is a flat and slightly flooded area with mature oak forest with many clusters of the giant terrestrial bromeliad, *Greigia sylvicola*. This valley is classified as Tropical Montane Rainforest (Holdridge 2000) with a short two month dry season beginning in December (Herrera 1985). We sampled the leaf litter, logs, mosses and bromeliads in several places through the trail searching for amphibians and reptiles. All the collected specimens were housed in the Zoology Museum of the Universidad de Costa Rica (UCR).

We followed Hedges *et al.* (2008) who assigned to the genus *Diasporus* those species included in the older *Eleutherodactylus diastema* group (Savage 2002). Terminology for morphological characters follows Savage (2002). All measurements of external morphology were recorded in mm to the nearest 0.1 mm with dial calipers and the aid of a dissecting microscope on preserved specimens. We report the measurements as mean \pm 1 standard deviation. The sex of the specimens was determined by observing the vocal sac and the vocal slits; only individuals with vocal sac distended were considered reproductive males, otherwise they were considered non-reproductive males; individuals lacking vocal slits were classified as females. That classification was corroborated by dissecting specimens to verify sex and gonadal development. For our species diagnosis, we followed the 14 numbered statements developed by Lynch and Duellman (1980). Color descriptions were based on photographs of the specimens in life or recently preserved.

Between 00:30 am and 01:00 am on July 11, 2008, the vocalizations of six adult males were recorded in the field before being collected, using a digital recorder Marantz PMD 660 and a Sennheiser Me 66 directional microphone at a distance no greater than 1 meter. Temperature during recording was measured using a mercury thermometer. We made all recordings in a high quality uncompressed format, using 16-bit linear Pulse Code Modulation PCM at a sampling rate of 44100 Hz. Analysis was performed with the software, Raven 1.2.1 (FFT Hanning Window 512; 50 % temporal overlap 86.1 Hz of grid spacing). In total, 56 notes consisting of a single pulse were analyzed. None of the calls were filtered for the analysis, because of their high quality. Range of frequencies, highest and lowest frequencies, dominant and fundamental frequencies, duration of the pulses, interval between pulses and pulse rate were determined by selecting directly on the oscillogram and spectrogram the areas of interest, then measurements were automatically provided by the software. The terminology for the vocal analysis follows that presented in Duellman (1970).

To evaluate the distribution range of the new species, we mapped the Altamira to Valle del Silencio route using data provided by Instituto Nacional de Biodiversidad (INBio) geographic information system. In this map we delimited areas in which we detected calling activity of the new species. In addition, we used the data from other herpetological surveys made close to the Valle del Silencio by the senior author and from the literature (Duellman & Myers 1980; Lips 1998) to define the maximum area of distribution. Using topographic map 1: 50 000 of the Valle del Silencio (Instituto Geográfico Nacional 1969) and the altitudinal and topographic features where this new species were seen or heard during field work, we determined a polygon for its potential distribution and calculate the area using ImageTool version 3.00 (UTHSCSA 2005-2000).

In order to establish conservation priorities for the new species, we assessed its environmental vulnerability score (EVS) analyzing its extent of geographic range, ecological distribution and degree of reproductive specialization. The initial score is three and represent species with generalize reproductive strategy, wide distribution and non threat by human activities. Final score varies from three to 17 been this the highest vulnerability value corresponding to species with highly restricted areas, very specific site requirements for oviposition and threat by commercial or non commercial use (Wilson & McCranie 1992; Wilson & McCranie 2004). We defined a conservation status for this species based on abundance, distribution and the protection of the habitat applied the IUCN criteria for species population status (IUCN 2001).

Molecular data of the new species were obtained by PCR and sequencing of part of the recombination activating protein 1 gene (*RAG-1*) of samples UCR 20491 (adult female), UCR 20496 (adult male) and UCR 20497 (juvenile male).

DNA extraction was performed using the Wizard Genomic DNA Purification Kit (Promega), from thigh muscle. Primers RAG1F (5'-ACATGCGACGTTTGCAATTC-3') and RAG1R (5'-TGTTGAGAGTATTTTCCAAGCAG) were designed using the Primer 3 software version 0.4.0 (Whitehead Institute for Biomedical Research, Steve Rozen, and Helen Skaletsky 1996-2007), based on the *Diasporus diastema* (probably *Diasporus hylaeformis* because in the locality reported for this specimen this is the species that occurs) sequence (EU186752) from the genomic on line database used the software Basic Local Alignment Search Tool (BLAST) version 2.0 (National Center for Biotechnology Information, National Library of Medicine and National Institutes of Health 2009) since it is the morphologically most related species. PCR was performed using the High Fidelity Enzyme Mix (Fermentas Life Science 2009) following the manufacturer's procedures and the following thermocycling profile: 2' at 94°, 30'' at 94°, 30'' at 60° to 50° (10 "touchdown" cycles -1°/min) and 1' at 72°. Then, 24 cycles of 30'' at 94°, 30'' at 50° and 1' at 72°. Finally, 10' at 72°. Amplification products were purified using the QIAquick PCR purification Kit (Qiagen 2009) and sequencing reactions in both directions were prepared using the BigDye terminator Kit version 3.1 (Applied Biosystems 2002) and read by an ABI3130 Sequencer (Applied Biosystems). The analysis was done in Sequencing Analyzer (Applied Biosystems) and Seqman II version 5.03 (DNASTAR 1989–1991) software. Comparison with orthologous genes was made using the nucleotide BLAST version 2.0 (National Center for Biotechnology Information, National Library of Medicine and National Institutes of Health 2009), and a molecular phylogenetic tree was reconstructed using CLUSTALW version 1.6 (EMBL/EBI 1996).

Results

Diasporus ventrimaculatus sp. nov.

Holotype. Male, UCR 20493; collected on July 10, 2008 by Gerardo Chaves, Adrián García, Guillermo Artavia and Grupo de Guías Locales de Biolley y Altamira in the Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica-Panama border (9.1116 N, -82.96172 W, 2550 m elevation).

Paratopotypes. Male: UCR 20494, UCR 20500, UCR 20503, UCR 20504, UCR 20505 UCR 20506; Female: UCR 20491 and UCR 20492. All material of these specimens were gathered by the same collectors and in the same place as the holotype.

Diagnosis. A member of the genus *Diasporus* based on the characteristic “dink” call, oval palmar tubercle, longitudinal gular folds in the edges of the deflated vocal sac and the presence of spadate disk covers on some fingers and toes. Members of this species have (1) skin on dorsum and venter areolate, but some individual are smooth; ventral disk absent; (2) tympanic membrane absent but tympanic annulus evident beneath skin, tympanum length is 50% of eye; (3) snout short, mucronate in dorsal view (truncate in females), truncate in profile; (4) upper eyelid lacking tubercles (but some individuals have few tubercles), as wide as interorbital distance (IOD); cranial crest absent; (5) vomerine odontophores absent (only present in the black male, mentioned below); (6) vocal slits large, external vocal sac present, nuptial pads absent; (7) first finger shorter than second, the last commonly with disk round, other fingers with disk spadate; (8) fingers broad, bearing narrow lateral fringes; subarticular tubercles round and flattened; palmar tubercle oval, round and flat, shorter than thenar tubercle; supernumerary tubercles not obvious (9) ulnar tubercle and fold absent; (10) heel and tarsus lacking tubercles and folds; (11) outer metatarsal tubercle flat and round; supernumerary tubercles not obvious (12) toes with lateral fringes; webbing absent; disk spadate (in juveniles slightly expanded); toe III reach the penultimate subarticular tubercle of toe IV; (13) light gray interorbital bar and black parietal area; dorsal head varies from cream to black; upper lip cream with small dark areas formed by small black dots; dorsum smooth red to pink in males, black with numerous small black dots in females; venter varies from red to pink in males and some of them with small white and black spots on ground color, otherwise females with large white blotches in a black ground; groin and underside area of limbs color vary according to the venter coloration in both sexes; posterior surfaces of thighs vary from red to black; dorsal limbs with diffuse transversal bars former by small dark dots, and in general these small black dots cover many areas of the body include bones; (14) SVL, in males 20.2 – 23.5 mm, in females 23.8 – 24.7 mm.

Holotype description. Adult male; body moderately robust; head about as wide as body, 116.7% wider than longer; head width 39.1% SVL; head length 33.5% SVL; snout round to truncate in dorsal view and truncate in profile; canthus slightly concave, softly sharp in profile; loreal region nearly flat; lips rounded; nostrils barely protuberant, directed laterally; width of upper eyelid less 81.6% of interorbital distance; eye large, its diameter much greater than its distance from nostril (123.8%); tympanum round with annulus visible beneath the skin, separated from eye by distance less than the tympanum diameter; supratympanic fold weak, running straight posteriorly, from point above tympanum, obscuring posteriodorsal edge of tympanum. Tongue small, transversally oval and it's free for about one third of its length; choanae small, oval, directed laterally and cover by the maxillae in ventral view; vocal slits present, large, longitudinal, lateral to base of tongue.

Forelimb slender; ulnar tubercles and fold absent; palmar tubercle low, round and about same size as thenar tubercle; subarticular tubercles large and rounded; supernumerary tubercles absent; fingers slender, with small lateral fringes; relative lengths of fingers I<II<IV<III; tips of fingers expanded, rounded, and spadate in most of them; nuptial pads absent. Hind limb moderately slender; tibia length 50.2% SVL; foot length 40.8% SVL; heel and tarsus lacking tubercles and folds; metatarsal tubercle round and large; subarticular tubercles large and rounded; supernumerary tubercles absent; toes slender, with lateral and small fringes; relative lengths of toes I<II<III<V<IV; tips of toes expanded, even round, spadate, with small

circumferential grooves. Skin on dorsum smooth; upper eyelids lacking tubercles; skin on venter areolate; discoidal fold not evident; skin ventral and ventrolateral to cloacae granular.

Coloration of holotype on preservative. Dorsum of head cream with diffuse darker brown spot to the anterior area; interorbital bar light gray and parietals are black, supratympanic folds outlined above and below by dark chocolate brown dots; dorsal surface of forelimbs slightly wither brown, with a small dark dots in the line of the wrist; dorsal surface of hind limbs similar to forelimbs with diffuse brown crossbars on shanks, thighs less colored but many small black dots; upper lip with diffuse brown bars; flanks lighter brown, changing ventrally and posteriorly to more red, with small white spots; groin red with small white spots and numerous small black dots; gular sac red with few and small black dots; ventral surfaces mottled with white spots on reddish ground color; iris pale brown.

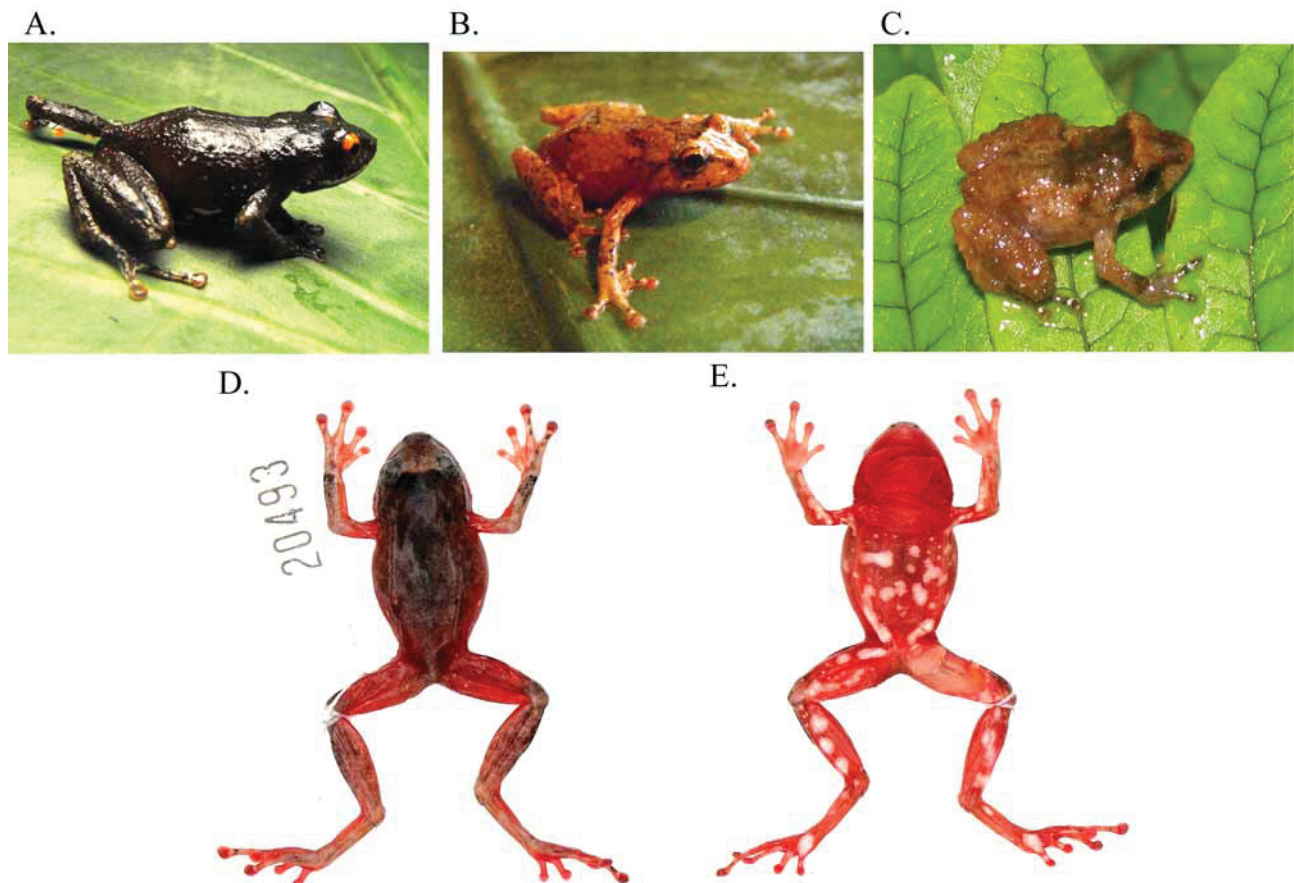


FIGURE 2. Photographs of a female (A), male (B) and juvenile (C) of *Diasporus ventrimaculatus* in life to show the sexual and ontogenetic and sexual polymorphism. A dorsal (D) and ventral (E) picture of the holotype in preservative is presented to show the white blotches distinctive of the new species.

Coloration of holotype in life (Fig. 2BDE). Dorsum orange, with a light gray interorbital bar black parietals that turns into darker diffuse color that extent near to the middle of the body. The anterior part of the head is slightly more yellow than the rest of the body, with a thin black line that starts in the snout, crosses the eye and ends at the tympanum. Dorsal surface of the limbs are orange with black spots in the forearms and some of the digits; black spots in the hindlimbs above a less colored surface. Flanks orange; groin red with small white blotches. Ventral surfaces of body and limbs are red with big and irregular white spots, vocal sac uniform red. A black ring delimited orange iris.

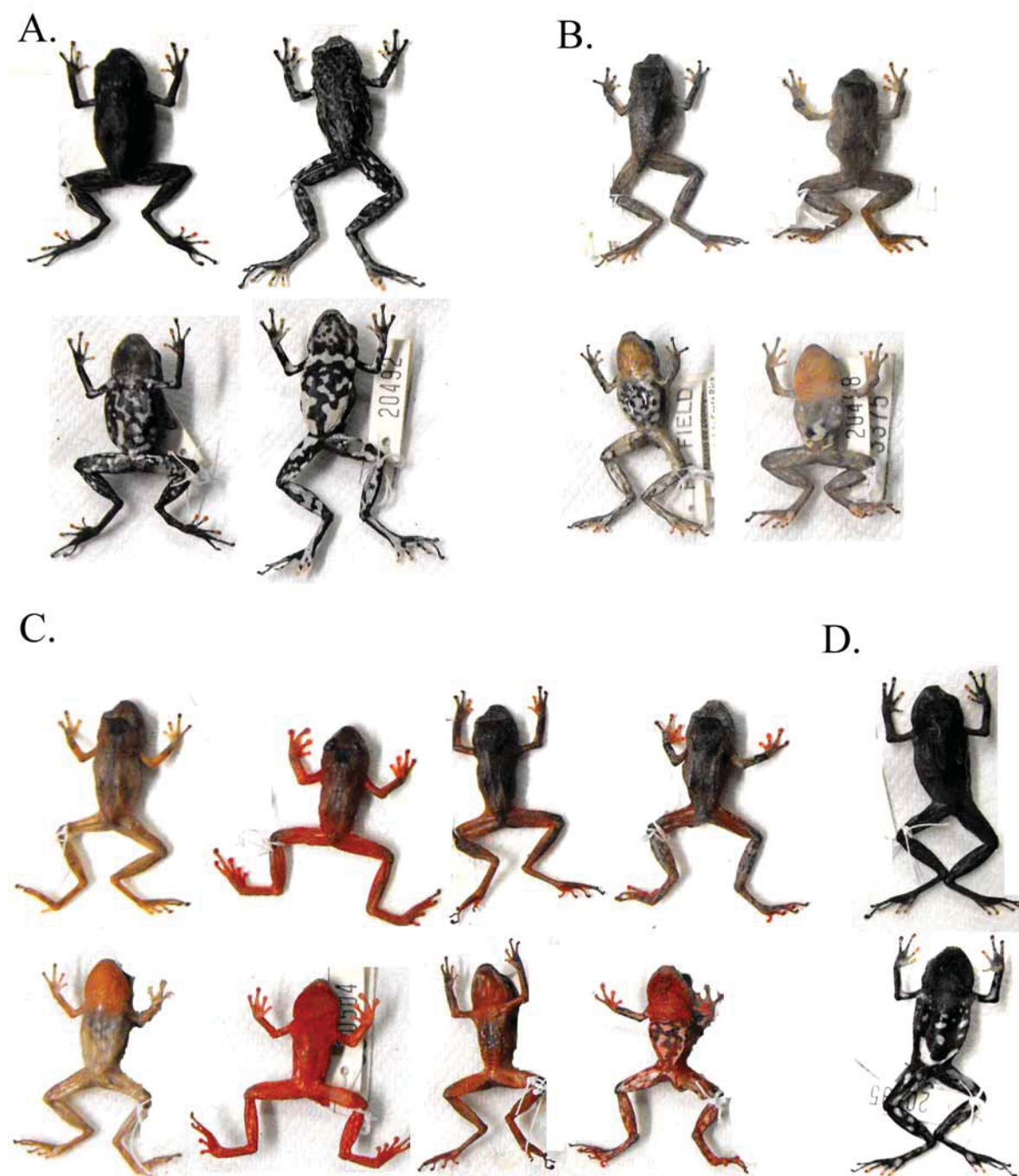


FIGURE 3. Dorsum and venter of females (A), non reproductive males (B), males (C) and black male (D), illustrating the large variation in color and patterns in the population of *Diasporus ventrimaculatus*. The black male shows the female pattern and coloration (see text).

Measurements of holotype (mm). SVL 23.3; tibia length, 11.7; head length, 7.8; head width, 9.1; interorbital distance 3.8; width of eyelid 3.1; diameter of eye 2.6; diameter of tympanum 1.3; fourth toe length 9.5.

Measurements of paratopotypes (mm). Measurements from six males and two females with mean and range in parenthesis. SVL males 21.8 ± 1.2 (20.2 – 23.5), females 23.9 ± 0.8 (23.2 – 24.7); tibia length, males 11.0 ± 0.7 (10.0 – 11.9), females 12.2 ± 0.0 ; head length, males 7.3 ± 0.3 (7.0 – 7.8), females 8.3 ± 0.1 (8.2 – 8.3); head width, males 8.4 ± 0.3 (7.9 – 8.7), females 9.5 ± 0.7 (9.0 – 10.0); interorbital distance, males 3.7 ± 0.2 (3.3 – 3.9), females 3.9 ± 0.3 (3.7 – 4.2); width of eyelid, males 3.2 ± 0.2 (2.9 – 3.8), females 3.9 ± 0.1 (3.8

– 3.9); diameter of eye, males 2.7 ± 0.2 (2.3 – 3.1), females 3.2 ± 0.1 (3.1 – 3.2); diameter of tympanum, males 1.3 ± 0.2 (1.0 – 1.8), females 1.5 ± 0.1 (1.4 – 1.5); fourth toe length, males 8.9 ± 0.6 (7.8 – 9.7), females 10.90 ± 0.42 (10.6 – 11.2).

Color polymorphism and sexual dimorphism. Males (Fig. 2B, 3C): dorsal head varies from cream to gray with a variable number and distribution of small black dots, a few individuals had red, brown or white blotches, all but one of the males present diffuse black and white bars in the upper lips; dorsum varies from red to brown with many small black dots that in some individuals form small dark blotches; groin red with small white blotches and small dark dots, but in some individuals are beige with or without white blotches; dorsal surface of thigh red with many brown blotches and small dark dots produce diffuse bars in most of the individuals; throat varies from orange to red with variable amount of small black dots; venter and underside thigh fluctuate from beige to red; all the individuals have small white blotches and have great variation in the number and distribution of the small black dots; iris red, but may be brass-colored in some individuals.

Black male (Fig 3D): Dorsum black, snout and flanks are darkish gray. Venter and underside area of the limbs are similar to the females. This is the only male with female coloration.

Non-reproductive males (Fig 3B): Head, snout, dorsum and tibia uniform reddish gray; interorbital bar light gray; throat similar to reproductive males; venter, groin and underside area of the thigh from beige to red with irregular large black and white blotches similar to females. Iris is brass colored.

Females (Fig 2A, 3A): head, snout, dorsum and tibia uniform darkish gray or with white and black blotches; venter, groin and underside area of the thigh are black with irregularly sized and irregularly distributed white blotches that are bordered with many small black dots; iris is red or brass-colored.

Juvenile (Fig 2C): SVL 18.3 ± 3.4 mm ($n = 4$). Head, snout, posterior area of dorsum and tibia uniform beige or with irregular yellow, red, brown and cream spots; interorbital bar yellow and parietals brown; throat, venter, groin and underside area of the thigh transparent with many small black dots and some beige and black blotches. Iris is brassy.

Hatchling: SVL 9.2 ± 2.0 mm ($n = 2$). Tip of the snout, interorbital bar and dorsum black separate with large white blotches. Flanks are black and white. Venter and underside area of the legs are whitish gray.

Molecular genetics. 364 nucleotides from the *RAG-1* gene could be compared between individuals UCR 20491 (adult female), UCR 20496 (adult male) and UCR 20497 (juvenile male). Four SNPs could be identified. Sequences UCR 20491 and UCR 20496 were 99.725% identical (only one difference), sequences UCR 20491 and UCR 20497 were 99.176% identical (three differences), whereas sequences UCR 20496 and UCR 20497 were 98.901% identical (four differences). All codons, from all individuals, codify for the same aminoacids (no missense mutation have been found): all SNPs found are non-coding. On the contrary, alignment between the new species' sequence and the *Diasporus hylaeformis* *RAG-1* sequence showed a transversion c.159 C>A (EU186752) that means an aminoacid difference Thr53Asn (ABY57667) between the two species. Phylogenetic tree based on the *RAG-1* sequences of these species and the most related ones found in the genomic on line database (BLAST), indicates that the new species and *D. hylaeformis* have a very close evolutionary history, and it is closer to the Caribbean genus *Eleutherodactylus* than to other mainland genus of direct development frogs such as *Craugastor* and *Pristimantis*.

Vocalization. The call structure is given in Fig. 4. The mean temperature during recording was 14 °C at 00:45 hours. Vocalizations produced by the holotype and other 5 males recorded and analyzed, are “dinks” similar to those of the other Costa Rican species of *Diasporus*; a sound reminiscent of a note from a xylophone. The call consists of monophasic notes that are repeated slowly in a range between 2140 and 2995 Hz (Fig 4A). The typical and dominant and fundamental frequencies (Fig. 4B) are the same, about 2550 ± 55 Hz (2495–2605) and there is no evident frequency modulation. Call duration (Fig 4B) is about $79.65 \text{ ms} \pm 8.90 \text{ ms}$ and interpulse interval $5.15 \text{ s} \pm 1.55$, with a call rate of approximately 11 pulses per minute.

Natural history. This species is found in many areas of the Valle del Silencio oak forest especially near clumps of terrestrial bromeliads. They can also be found in different layers of the forest, from canopy to less than half meter above the ground. We never observed them on the ground. Most males call inside the vegetation on the upper surfaces of the leaves, branches and stalks from both arboreal and terrestrial

bromeliads and trees, but some individuals were exposed on the leaves close to the trails. All of the females were found in the vegetation, at least at 50 cm height, in generally lower than the males.

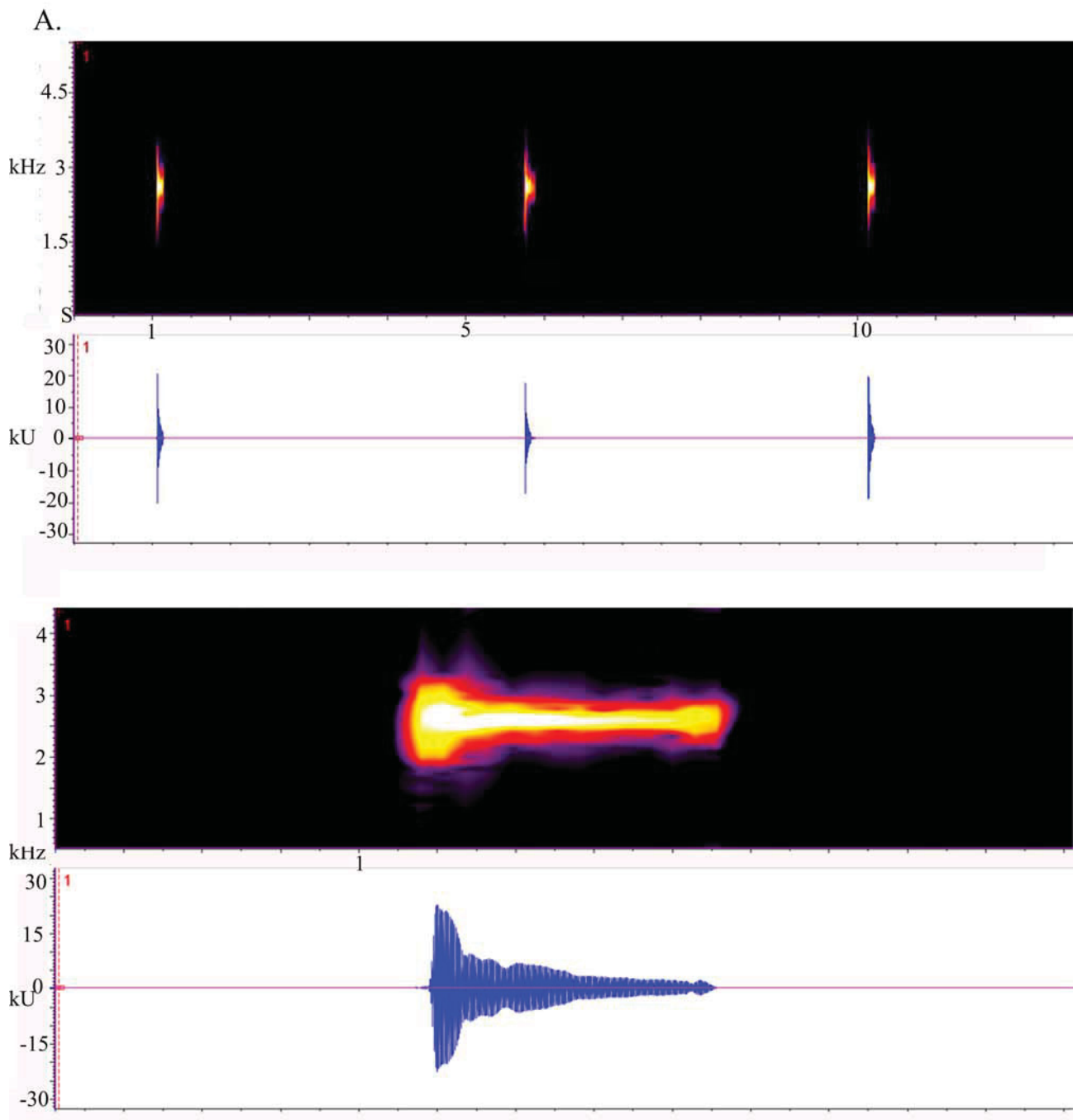


FIGURE 4. Spectrogram and oscillogram of the mating call of *Diasporus ventrimaculatus* **sp nov** recorded at Valle del Silencio, Cordillera de Talamanca, Costa Rica. A. Temporal parameters B. Detail of spectral parameters in a single pulse.

We did observe several males, females and juveniles of various sizes emerging in the leaf litter trapped between the leaves of the giant terrestrial bromeliad, *Greigia sylvicola*, in the beginning of the night (between 6 to 7 o'clock). We estimate that we heard hundreds of calling males in one particular small area of forest, where it was common to find more than one male perched in the same plant. Males start to call in the evening (18:00 to 19:00) until late in the morning (5:00 to 6:00) and can occasionally be heard during the day but in less frequency and abundance. On sunny days fewer males call than during rainy days and most of them call close to the flooded areas.

Distribution. On the trail between Altamira and Valle del Silencio (1300 to 2600 m), we found this species to occur around 2500 m of altitude. and only in the Valle del Silencio. We never detected *D. ventrimaculatus* on the slopes of the valley but only in the flat area. We did not detect individuals along a 3 km trail that runs along the east side of the Río Terbi at the base of Cerro Asidbeta, this trail it's only 15 meters from the holotype place but in the opposite margin of the river. Furthermore, this species was not present in the Jardín (9.1298 N, -82.9673 W, 2450 m), a larger open moss-backed area with numerous treelike ferns (fog) located on the northeastern part of the valley. Near Cerro Bekón (9.1594 N, -83.0478 W, 2600 m), 11 km to west of the Valle del Silencio and at the same altitude, the senior author never saw any individuals of the new species during a survey in December 2007, in spite of surveying more than one hundred terrestrial bromeliads.

Numerous surveys were conducted in this region in the late 20th century by noted herpetologists including William Duellman, Charles W. Myers, Douglas Robinson, Federico Bolaños, Karen Lips, David Wake and others (Duellman & Myers 1980; Lips 1998; Federico Bolaños personal communication). These surveys included Cerro Pando (8.9250 N, -82.7269 W, 2400 m), Mellizas (8.8895 N, -82.7775 W, 1400 m), Sitio Las Tablas (8.9361 N, -82.7528 W, 1880 m), Alturas de Cotón (8.9392 N, -82.8390 W, 1400 m) and Cerro Echandi (9.0340 N, -82.8246 W, 3100 m) and in spite of these past surveys *D. ventrimaculatus* was not reported or collected at these localities. In the 1980's in a survey carried out by the courageous Costa Rican botanist Luis Diego Gómez to Cerro Bine (9.0950 N, -82.8929 W, 3242 m), at the headwater of the Río Terbi, he not registered this species there, although he did collect an important new species of salamander there (Federico Bolaños personal communication).

Based on these historic surveys we infer that this species probably does not occur outside of the southeastern region of the Valle del Silencio, specifically in the flat and flooded area of around 2.5 km² in the west side of the Río Terbi (Fig. 1), enclosed by the Hoffmann, Sin Nombre, Turubo and Adsibeta peaks and the moss-backed area of the Jardín.

Conservation status. We calculated an EVS score of 17 for *Diasporus ventrimaculatus* that indicates a maximum conservation priority. In spite of the abundance of *Diasporus ventrimaculatus* and the good conservation condition of its habitat, we believe that this high EVS value and its reduced geographical extension of less than 3 km² put this species in a critical endanger category under the IUCN criterion B2aciv. We hypothesize that the number of adult reproductive individuals would fluctuate very much in a small population and there are several examples of drastic amphibian population declines in other species with a reduced or small range of distribution in Costa Rica (see below for details in the discussion).

Etymology. The specific name *ventrimaculatus* is a composed term derived from the Latin words *ventris* meaning venter and *maculatus* meaning spotted. The name is in relation to the larger dark and white blotches in the venter found in most of the individuals and mainly in the females, this coloration is not found in any other species of the genus.

Discussion

Cordillera de Talamanca is a place of high endemism for amphibians particularly for salamanders (Wake 2005), but there are numerous endemic anuran species known in this region (Savage 2002). Endemic amphibians commonly have a range of only a few square kilometers (García-Paris *et al.* 2000; Savage 2002) and this increases the risk of extinction due that the populations themselves are small (Begon *et al.* 2006). The total area in where *D. ventrimaculatus* is known to inhabit occurs in the Valle del Silencio and is approximately 1500 hectares. The Valle del Silencio is unique within the known areas of Talamanca because it encloses particular habitats. It presents a large river, a flooded oak forest mixed with bamboo and giant terrestrial bromeliads surrounded by high peaks and the only flat area is vegetated with a rare formation of moss and ferns.

Diasporus ventrimaculatus differs from all other species of the genus by the presence of several black and

white blotches in the venter and the formidable ontogenetic and sexual polychromatism. All other species in Costa Rica have reddish cream or pale yellow venter with few small black dots. Outside of Costa Rica, only *D. anthrax* and some individuals of *D. quidditus* have black ventral coloration, but never with large white blotches (Lynch 2001). Characteristic “dink” advertisement call and the red dorsum coloration relate this new species with *D. hylaeformis*.

We found that the voice of *D. ventrimaculatus* differs in both spectral and temporal parameters when it is compared with *D. diastema* (Fouquette 1960; Savage 2002). *Diasporus diastema* has a modulation in the call that is not present in the new species. In addition *D. diastema* calls in higher frequencies (3100 to 4000 Hz), has a longer call duration (205 ms) and a higher call rate (30 to 40 pulses per minute) than *D. ventrimaculatus*. Although the call of *D. hylaeformis* is not published yet, one of the authors (AG-R) had recorded and analyzed several populations of it in Costa Rica. From this current study we determined that the new species calls in a frequency range similar to *D. hylaeformis* (2355 to 3055 Hz), but the call duration in this later species (214 ms \pm 21, n = 86) is on average longer than in *D. ventrimaculatus*.

The *RAG-I* gene sequences suggests that adult male (UCR 20496) and female (UCR 20491), as well as a juvenile male (UCR 20497) belong to the same species. Genetic analysis of this gene and its codified protein indicate that *Diasporus ventrimaculatus* is closely related to *D. hylaeformis*, conforming a separated group from the *Eleutherodactylus* genus. This result is in agreement with our morphological data. The relation of both species with the Antillean clade of “eleutherodactilines” is according with recent genetics analysis of the group (Hedges *et al.* 2008).

The bright red coloration on the dorsum and dorsal surfaces of the arms and legs of male *Diasporus ventrimaculatus* is not found in any other species of the genus where their colors tend to be yellow cream, although *D. hylaeformis* has red phases in some populations (Savage 2002, personal observation). The ventral color pattern of white blotches on a red (males) or black (in females) ground color is similar to other anuran species that exhibit “flash colors” that are hidden when the animal is quiet and appear abruptly when the animal is perturbed (Wells 2007). For example, white blotches in the groin and underside of the legs are common in treefrogs (Duellman & Trueb 1986) and some *Pristimatis* species such as *P. altae*, *P. cruentus*, *P. pardalis* (Savage 2002).

Polychromatism is very common in *Eleutherodactylus sensu lato* species (Hoffman & Blouin 2000; Savage & Emerson 1970) and in this regard, *D. ventrimaculatus* presents a striking condition. Ontogenetically changes in coloration are common in amphibians, but normal adult color pattern is reached within a few days or weeks (Duellman & Trueb 1986). Hoffman and Blouin (2000) report similar cases in only 39 out of 225 species, most of them in the genus *Hiperolius*. *Diasporus ventrimaculatus* differs in this respect because all the phases of development are different. Even rarer in amphibians is dichromatism between the sexes that involve more than the vocal sacs or throat area, where males becoming black during the breeding season (Duellman & Trueb 1986). Sexual dichromatism is known for 28 out of 228 polychromatic frogs (Hoffman & Blouin 2000), and 6 of these species belong to the *Eleutherodactylus sensu lato* genus. For Costa Rican species of amphibians *Diasporus ventrimaculatus* sexual dichromatism is only compared with that present in *Insilius (Bufo) periglenes* where the males were bright orange and the females were cryptic olive/black with red spots (Savage 1967). The causes and functions of the sexual dichromatism in amphibians are unknown (Duellman & Trueb 1986), but some authors propose sexual niche-partitioning (Shine 1989), such that differential predation risk between sexes would influence color variation (Hayes 1997).

Our observations indicates that *D. ventrimaculatus* is an arboreal species like all the species in the genus (Hedges *et al.* 2008). The use of the terrestrial bromeliads to rest and call is not reported for any species of this group, where only arboreal bromeliads are used commonly (Savage 2002). The use of all possible habitats in the valley with the exception of the ground for this new species can indicate lower competition with other species in the area; that is reinforced by the fact that only three species of salamanders and one species of leaf litter frog (*Craugastor podiciferus*) were reported in the forest of the valley (other species of frogs were recorded from El Jardín in the north area of the valley).

Since 2002 Costa Rican herpetologists have been declared three endemic frog species to be extinct:

Insulius periglenes, *I. holdridgei* and *Craugastor escoces* (IUCN 2008). Very little ecological information about these species (Novak & Robinson 1975; Savage 1967) was collected before they disappeared, and today their extinction is declared enigmatic (Bruce *et al.* 2004). As far as was known all of these species had small, restricted ranges.

For these reasons, it is very important continue surveys on *D. ventrimaculatus* to evaluate its population status and to detect long-term population trends. Investigating dispersal and population genetics can also be a useful tool to evaluate aspects of this species biology that can be used for conservation management. An important issue to the authors is to develop a comprehensive management plan to regulate tourist visitation and to promotion of scientific research in the area to ensure better conservation strategies for this species and its habitat. An integral component of this plan would be for the participation of the local communities, government institutions, universities and non-governmental organizations in the area (Asociación de Productores de la Amistad (ASOPROLA) and Asociación de Mujeres Organizadas de Biolley (ASOMOBI)) is very important to achieve these goals.

Material revised

Female UCR 20533; subadults males UCR 20497, UCR 20498; juveniles UCR 20531, UCR 20534; new born UCR 20502, UCR 20535. Ten more specimens were only photographed for analyzing polychromatism. All of them were collected in the same place of the holotype and paratypes.

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